

## 6. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting, measuring, and/or monitoring vinyl chloride, its metabolites, and other biomarkers of exposure and effect to vinyl chloride. The intent is not to provide an exhaustive list of analytical methods. Rather, the intention is to identify well-established methods that are used as the standard methods of analysis. Many of the analytical methods used for environmental samples are the methods approved by federal agencies and organizations such as EPA and the National Institute for Occupational Safety and Health (NIOSH). Other methods presented in this chapter are those that are approved by groups such as the Association of Official Analytical Chemists (AOAC) and the American Public Health Association (APHA). Additionally, analytical methods may be included that modify previously used methods to obtain lower detection limits and/or to improve accuracy and precision.

### 6.1 BIOLOGICAL SAMPLES

The analytical method used to analyze for the presence of vinyl chloride in biological samples is separation by gas chromatography (GC) combined with detection by mass spectrometry (MS), flame ionization detector (FID), or electron capture detector (ECD). Vinyl chloride and/or its metabolite, thiodiglycolic acid, have been detected in breath, urine, blood, and tissues. Breath samples can be concentrated by cryogenic trapping. The two methods most commonly used to prepare liquid and solid samples are concentration by a purge-and-trap technique or headspace analysis. Concentration not only increases the sensitivity but, in certain instances, may decrease the sample separation time prior to quantitation. Details of commonly used analytical methods for several types of biological samples are presented in Table 6- 1.

Vinyl chloride was determined in exhaled air by concentration with a multistage cryogenic trapping system followed by thermal desorption using GC/FID, GC/ECD, and GC/MS (Conkle et al. 1975). Sensitivity is in the low-ppb range. The authors of this study noted that the reproducibility of the subject/sampling system was inconclusive; a larger experimental population is needed for its demonstration. The quantitative data reflected considerable scatter, apparently indicating the

**TABLE 6-1. Analytical Methods for Determining Vinyl Chloride in Biological Samples**

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Breath	Breath collected in pipets lined with Saran® film; direct injection into gas chromatograph	GC/FID	NR	NR	Baretta et al. 1969
Breath	Cryogenic trapping of expired air; thermal desorption into gas chromatograph	GC/FID, GC/ECD, and GC/MS	NR	NR	Conkle et al. 1975
Urine	Acidified and desiccated overnight; add methanol; derivatize with diazomethane; add ion-exchange resin	GC/MS	50 ng/mL	NR	Muller et al. 1979
Urine	Internal standard added to urine; acidification and ethyl acetate extraction; evaporation of solvent; addition of <i>N</i> -trimethylsilyl-diethylamine in pyridine (1:1); injection into gas chromatograph	GC/FID, GC/MS	10 mg/L	NR	Draminski and Trojanowska 1981

**TABLE 6-1. Analytical Methods for Determining Vinyl Chloride in Biological Samples (*continued*)**

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Blood and tissues	Extraction in ethanol-water mixture, incubation, injection into gas chromatograph	GC/FID	5 ng/mL blood	75–79% blood	Zuccato et al. 1979
	Tissue preparation also includes freezing and homogenization before the extraction procedure		30 ng/g tissue	76–92% tissue	

GC/ECD = gas chromatography/electron capture detector; GC/FID = gas chromatography/flame ionization detector; GC/MS = gas chromatography/mass spectrometry; NR = not reported

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variability of the biological system and the trace amounts of the compound. The additional requirement for long-term coupling (30-60 minutes) of the sampling system to the subject probably limits the method to industrial health applications, with relatively robust subjects (Conkle et al. 1975). Baretta et al. (1969) monitored exposure to vinyl chloride by breath analysis. The breath samples were collected in pipets with plastic caps lined with six layers of Saran film identical to that used for the construction of Saran air sampling bags. Aliquots were drawn from the pipets and injected directly into a gas chromatograph equipped with FID. One limitation of this method is its reduced ability to detect vinyl chloride when air concentrations in the workplace are below 50 ppm.

Vinyl chloride has been measured in rat blood and tissues using headspace GC/FID (Zuccato et al. 1979). In headspace analysis, the gaseous layer above the sample is injected into the gas chromatograph. Sample preparation steps for rat blood and tissues involve extraction in an ethanolwater mixture, incubation, and direct injection into the gas chromatograph. Sample preparation for tissues includes an extra step involving freezing and homogenization before the extraction procedure. The recovery ranged from about 75% to 92%. The method is sensitive to 5 ng/mL vinyl chloride in blood and 30 ng/g in tissues.

Muller et al. (1979) employed GC/MS as a selective biomonitoring method for the quantitative measurement of thiodiglycolic acid, a urinary metabolite of vinyl chloride. They reported a sensitivity of 50 ng/mL. Precision was generally good. These investigators noted that some thiodiglycolic acid has been found in supposedly unexposed subjects. Therefore, exposure to low levels of vinyl chloride could be masked by background metabolic levels within normal limits. This may limit the application of biological monitoring for the measurement of vinyl chloride following low-level exposure (Muller et al. 1979; van Sittert and de Jong 1985). In a study by Jedrychowski et al. (1984), urinary excretion of thiodiglycolic acid was determined using GC/FID. The urine was extracted twice with ethyl acetate prior to analysis. No recovery data were given for this method.

### 6.2 ENVIRONMENTAL SAMPLES

Analysis of environmental samples is similar to that of biological samples. The most common methods used to detect vinyl chloride in environmental samples are GC/MS, GC/ECD, and GC/FID. Concentration of samples is usually done by sorption on solid sorbent for air and by the purge-and-trap method for liquid and solid matrices. Alternatively, headspace above liquid and solid

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samples may be analyzed without preconcentration. Details of commonly used analytical methods for several types of environmental samples are presented in Table 6-2.

The primary method of analyzing vinyl chloride in air is GC combined with either MS, ECD, or FID. Air samples are usually pumped through a sample collection column with Tenax-GC, coconut activated charcoal, or spherocarb (a carbon molecular sieve material) as the most common adsorbents. Several authors have noted that Tenax-GC displays poor retention for vinyl chloride when the compound is present in the very low-ppb range (Bozzelli and Kebbekus 1979; Krost et al. 1982; McMurry and Tarr 1978). Vinyl chloride is thermally desorbed from the collection column and concentrated on a cryogenic trapping column located on the gas chromatograph. Vapors are heat released from the trapping column directly to the gas chromatograph (Bozzelli and Kabbekus 1979; Krost et al. 1982). Grab samples of air can also be obtained and preconcentrated on a cryogenic column (Rasmussen et al. 1977). The limit of detection for GC/MS and GC/ECD is in the sub-ppb range (Bozzelli and Kebbekus 1979; Harsch et al. 1979; Krost et al. 1982; Rasmussen et al. 1977). Accuracy is generally good (Bozzelli and Kebbekus 1979). With careful technique, precision is adequate, ranging from 5% to 20% (Bozzelli and Kebbekus 1979; McMurray and Tarr 1978).

Trace amounts of vinyl chloride in air and water were detected employing GC/ECD after derivatization to 1,2-dibromochloroethane (Wittisiepe et al. 1990, 1993). Air samples were taken by drawing a known volume directly through an ice-cooled adsorption tube. Water samples were purged with an inert gas before being drawn through the adsorption tube. The tubes were eluted with carbon disulfide, and the vinyl chloride was derivatized with bromine water to form 1,2-dibromochloroethane. This derivatization technique is used for enhancement of sensitivity with GC/ECD. The derivative was determined by capillary GC with ECD. The detection limits for air and water samples are  $50 \mu\text{g}/\text{m}^3$  and  $0.4 \text{ ng/l}$  (0.4 parts per trillion), respectively. Results from recovery experiments with dosed water indicated that accuracy was good.

Vinyl chloride can be detected in drinking water, groundwater, waste water, and leachate from solid waste. Analysis of vinyl chloride is done by purge-and-trap or headspace GC. The primary analytical method is separation by GC combined with MS, ECD, FID, Hall's electrolytic conductivity detector (HECD), or another type of halogen specific detector (HSD). In most methods, vinyl chloride is

**TABLE 6-2. Analytical Methods for Determining Vinyl Chloride in Environmental Samples**

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Occupational air	Vinyl chloride in air adsorbed in activated carbon trap and desorbed by carbon disulfide	GC/FID	0.04 µg per sample	94% at 1–64 mg/m <sup>3</sup> (0.00039–0.025 ppb)	NIOSH 1994a
Ambient indoor and outdoor air	Air containing vinyl chloride passed through activated carbon trap and desorbed by dichloromethane or carbon	GC/FID	5 ppb	NR	IARC 1978
Air	Adsorption on Tenax®–GC, or SKC® Carbon, then thermal desorption	GC/MS	0.33 ppb	NR	Krost et al. 1982
Air	Air prefiltered by Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> -treated glass fiber filter was passed through spherocarb adsorbent cartridge and thermally desorbed	GC/FID, GC/MS, GC/ECD	0.005 ppb	NR	Harkov et al. 1983, 1984

**TABLE 6-2. Analytical Methods for Determining Vinyl Chloride in Environmental Samples (*continued*)**

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Automobile exhaust	Exhaust samples contained in aluminized plastic bags	GC/FID	0.02 ppm	NR	Hasanen et al. 1979
Air	Trapped in cold Tenax®-GC trap; thermal desorption	GC/FID	NR	89.6% at 6 ppb; 100% at 60 ppb	Ives 1975
Air	Sample collected in pressurized canister is passed through a freezeout loop and subsequently heated	GC/ECD	0.01 ppb	NR	Harsch et al. 1979; Rasmussen et al. 1977
Air	Sample collected in polyester-coated plastic bags concentrated by freezeout and subsequently heated	GC/FID	0.4 ppb	NR	McMurray and Tarr 1978
Drinking water	Samples collected in serum reaction bottles; purge and trap technique	GC/HSD, GC/MS	NR	NR	Dressman and McFarren 1978

**TABLE 6-2. Analytical Methods for Determining Vinyl Chloride in Environmental Samples (*continued*)**

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Drinking water and waste water	Purge and trap in Tenax®-GC; thermal desorption	GC/HSD, GC/MS (EPA Methods 601 and 624)	0.18 ppb (HSD)	102% at 0.8–32.3 ppb	APHA 1985; EPA 1982d
Groundwater, liquid, and solid matrices	Purge at 45°C and trap in Tenax®-GC; thermal desorption	GC/HSD (EPA Method 8010)	0.18 ppb	102% at 0.82–32.3 ppb	EPA 1982e
Water	Purge into Carbosieve™ S III; desorption with CS <sub>2</sub> and bromine derivation	GC/ECD	0.0004 ppb	98.9% at 0.00625–62.5 ppb	Wittsiepe et al. 1993
Drinking water	Purge and trap in Tenax®-GC; thermal desorption	GC/Hall detector, GC/PID (EPA Methods 502.2 and 524.2)	0.04 ppb (Hall detector); 0.02 ppb (PID)	100–119% at 5–10 ppb	Reding 1987
Migration of monomer into drinking water from PVC pipes	Small sections put in water in sealed serum vial for a number of days at 20°C; solution directly injected into gas chromatograph	GC/FID	NR	NR	Ando and Sayato 1984

**TABLE 6-2. Analytical Methods for Determining Vinyl Chloride in Environmental Samples (*continued*)**

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Water	Sample in sealed vial is equilibrated at constant temperature; headspace gas injected into gas chromatograph	GC/FID	<1 ppb	NR	IARC 1978
Landfill gas	Gas from landfill sites sampled by PTFE tubing inside drive-in piezometers was adsorbed in Tenax®-GC or Porapak®, a sorbent; trapped sample desorbed and concentrated in liquid N <sub>2</sub> -cooled loop and flash desorbed	GC/MS	0.04–0.8 ppm	NR	Young and Parker 1984
Sediment and oyster	Homogeneous sample mixed with water and vinyl chloride purged into a closed loop injected into gas chromatograph	GC/ECD	2 ng/g (sediment); 4 ng/g (oyster)	NR	Wang et al. 1985

**TABLE 6-2. Analytical Methods for Determining Vinyl Chloride in Environmental Samples (*continued*)**

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Landfill gas	Sample collected in 2-L evacuated glass bulb; gas directly injected into gas chromatograph	GC/FID	NR	NR	Wood and Porter 1987
Food (orange drink, wine, olive oil)	Sample sealed in vials and equilibrated at 40°C for 2 hours; injected into gas chromatography	GC/FID	NR	NR	Chudy and Crosby 1977
Foodstuffs	Sample sealed in vials and equilibrated at 40°C for a minimum of 2 hours; headspace gas injected into gas chromatograph	GC/FID	1–5 ppb	NR	IARC 1978

CS<sub>2</sub> = carbon disulfide; EPA = Environmental Protection Agency; GC/ECD = gas chromatography/electron capture detector; GC/FID = gas chromatography/flame ionization detector; GC/HSD = gas chromatography/halogen specific detector; GC/MS = gas chromatography/mass spectrometry; GC/PID = gas chromatography/photoionization detector; HSD = halogen specific detector; N<sub>2</sub> = nitrogen; Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> = sodium thiosulfate; NR = not reported; PID = photoionization detector; PTFE = polytetrafluorethylene; PVC = polyvinyl chloride

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liberated from the liquid matrix by purging with an inert gas and concentrated by trapping on a suitable solid sorbent. Vinyl chloride is thermally desorbed and backflushed onto the column of the gas chromatograph with an inert gas. Detection of vinyl chloride is generally achieved using HECD, HSD, or MS (APHA 1985; EPA 1982d, 1982e; IARC 1978; Reding 1987). The limit of detection is in the sub-ppb range for halogen specific detectors (APHA 1985; EPA 1982d, 1982e) and in the low ppb range for MS (EPA 1982d). Accuracy is greater than 98% and precision ranges from 11% to 25% for GC/HECD and GC/MS (EPA 1982d).

EPA has made improvements in methods for measuring volatile organic chemicals. The major change is the use of smaller sample volumes allowed by increased use of capillary gas chromatographic columns. Capillary columns provide better resolution, minimum detection limits, and less column bleed than packed columns (Reding 1987).

Vinyl chloride has been measured in sediment using GC/ECD with sensitivity in the low-ppb range. Accuracy and precision data were not provided in the report (Wang et al. 1985). No information on analysis of vinyl chloride in soil was located. GC/HSD of headspace gases is the EPA-recommended method for solid matrices with sensitivity in the sub-ppb range. Accuracy (101.9%) is good and precision (11.4%) is adequate (EPA 1982d). Vinyl chloride levels in food have been determined using GC/FID. GC analysis by headspaces gases is a common method for testing foods, with sensitivity in the low-ppb range (IARC 1978).

### 6.3 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of vinyl chloride is available. Where adequate information is not available, ATSDR, in conjunction with the NTP, is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of vinyl chloride.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean

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that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

### 6.3.1 Identification of Data Needs

#### Methods for Determining Biomarkers of Exposure and Effect

**Exposure.** Methods are available for measuring vinyl chloride and/or its metabolite, thiodiglycolic acid, in breath, urine, blood, and tissue (Baretta et al. 1969; Conkle et al. 1975; Draminski and Trojanowska 1981; Muller et al. 1979; Zuccato et al. 1979). These methods are sensitive for measuring levels at which health effects might occur, and for measuring higher background levels that might be found in specific populations known to be exposed to elevated levels of vinyl chloride (e.g., workers in the plastics industry and individuals living in the vicinity of hazardous waste sites). Measurement of urinary thiodiglycolic acid can be used as an indicator of vinyl chloride intake as long as individual variability in metabolism (due to such factors as liver disease, use of drugs, and alcohol intake) can be accounted for (Hefner et al. 1975b; Muller et al. 1979). Exposure to vinyl chloride at concentrations below 1-5 ppm could be masked by background metabolic levels of thiodiglycolic acid within normal limits (Muller et al. 1979). Also, the formation of thiodiglycolic acid is not unique to vinyl chloride exposure (Norpoth et al. 1986; Pettit 1986). The methods are generally reliable, although increased precision for most methods would increase reliability. Background levels for the general population are ill defined (EPA 1985b). Further research on the relationship between low-level exposure and levels of vinyl chloride in biological media would be helpful in assessing the risks and health effects of chronic, low-level exposure.

**Effect** Existing methods are sensitive for measuring levels of vinyl chloride and its metabolite, thiodiglycolic acid, in individuals affected by exposure to very high levels of vinyl chloride (Baretta et al. 1969; Conkle et al. 1975; Draminski and Trojanowska 1981; Muller et al. 1979; Zuccato et al. 1979). Also, methods are available to detect DNA adducts produced by the reaction of vinyl chloride metabolites with DNA (Eberle et al. 1989; Young and Santella 1988). These DNA adducts are specific indicators of vinyl chloride's genotoxic potential. These methods, however, are not sufficiently sensitive to determine the genotoxic effects resulting from low-level exposure. Correlations between levels detected in biological tissues and fluids and specific observed effects for lower levels of exposure have not been established. Additional research in this area would allow

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better assessment of existing methods and would help in defining areas in which improvements are needed.

### **Methods for Determining Parent Compounds and Degradation Products in**

**Environmental Media.** Existing methods for determining vinyl chloride in air (Harkov et al. 1983, 1984; Harsch et al. 1979; Hasanen et al. 1979; IARC 1978; Ives 1975; Krost et al. 1982; McMurry and Tarr 1978; NIOSH 1994a; Rasmussen et al. 1977) and water (Ando and Sayato 1984; APHA 1985; Dressman and McFarren 1978; EPA 1982d, 1982e; IARC 1978; Reding 1987), the media of most concern for human exposure, are sensitive, reproducible, and reliable for measuring background levels in the environment. Research investigating the relationship between levels measured in air and water and observed health effects could increase our confidence in existing methods and/or indicate where improvements are needed. Methods specifically relating to the analysis of vinyl chloride in soils were not located. EPA does, however, have sensitive and reliable methods for determining the concentration of vinyl chloride in soil matrices (EPA 1982e), which include contaminated soils.

### **6.3.2 On-going Studies**

James Swenberg (University of North Carolina at Chapel Hill), in his work on DNA adducts as biomarkers of exposure and effect, is developing ultrasensitive methods with high specificity to quantitate the dose of vinyl chloride reaching DNA (FEDRIP 1994).

